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Synthesis of novel amphiphilic star-shaped poly(ε-caprolactone)-*block*-poly(*N*-(2-hydroxypropyl)methacrylamide) by combination of ring-opening and chain transfer polymerization

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Abstract

Novel amphiphilic star-shaped block copolymer, star-poly(ε-caprolactone)-*block*-poly(*N*-(2-hydroxypropyl)methacrylamide), was synthesized and characterized. Star-block copolymers with varying feed ratios were prepared by free radical polymerization of *N*-(2-hydroxypropyl)methacrylamide in the presence of a novel biodegradable, macromolecular chain-transferring agent star-poly(ε-caprolactone)-tetrakis-thiol. All star-block copolymers self-assembled in aqueous solutions to form unimodal micelles of 100–150 nm size. Micelle size was independent of polymer concentration. The critical aggregation concentrations of the polymers were in the range of 1–2.5 mg/l. The partition equilibrium constant of pyrene in the hydrophobic core of micelles was comprised between 5 and 8×10^5. The star-block copolymer micelles were loaded by a dialysis procedure with 5–12% (w/w) of a model poorly water-soluble drug (indomethacin). These star-block copolymers could prove useful as nanocarriers for the solubilization and transport of hydrophobic drugs. Star-shaped macromolecular chain-transferring agent reported in this work can also find application in the synthesis of a variety of novel biodegradable star-block copolymers. © 2002 Published by Elsevier Science Ltd.

Keywords: Amphiphilic; Star-block copolymers; Biodegradable chain-transfer agent

1. Introduction

Amphiphilic star-shaped block copolymers have recently attracted much attention because these polymers can behave as unimolecular micelles or be designed to exhibit a very low critical aggregation concentration (CAC) [1–3]. Star polymer exhibit smaller hydrodynamic radius and lower solution and melt viscosities as compared to the linear polymer of same molecular weight and composition [4–6]. Moreover, multiple polymer end groups could be functionalized for a variety of applications. These features are very attractive for medical use such as micellar drug delivery [7–9] and thermoplastic hydrogels [10]. So far, several amphiphilic star-block copolymers have been synthesized including star-poly(ethylene oxide)-*block*-poly(styrene) [11], star-poly (methyl vinyl ether)-*block*-poly(isobutylene) [12], star-poly(2,3-dihydroxypropylacrylate)-*block*-poly(methyl methacrylate) [13], star-poly(ethylene glycol)-*block*-poly (isobutylene) [14] and star-poly(methacrylic acid)-*block*-poly(isobutylene) [15]. However, star-block copolymers comprising hydrophobic biodegradable and hydrophilic biocompatible segments are of particular interest, especially for biomedical applications.

Choi et al. [16] synthesized star-poly(ethylene oxide)-*block*-poly(L-lactic acid) (star-PEO-*b*-PLA) and star-PEO-*block*-poly(ε-caprolactone) (star-PEO-*b*-PCL) by initiating ring-opening polymerization of L-lactide and ε-caprolactone, respectively, with four and eight arm PEO at 110 °C in the bulk. Li and Kissel [17] reported four and eight arm star-PEO-*b*-PLA, star-PEO-*b*-PLGA by ring-opening polymerization of L-lactide and L-lactide/glycolide, respectively, initiated with star-PEO in toluene at 70 °C, using triethylaluminum as catalyst. Floudas et al. [18] reported triarm star-PEO-*b*-PCL by two successive initiations of anionic polymerizations. Star-PEO-*block*-poly(γ-benzyl L-glutamate) (star-PEO-*b*-PBLG) was reported by Jeong et al. [9] by polymerization of *N*-carboxyanhydride of γ-benzyl-L-glutamate in dichloromethane at room temperature, initiated with bis-PEO-bis-amine. Unimolecular polymeric micelles were reported by Liu et al. [19] wherein, three mucic acid molecules in the central core were derivatized

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with fatty acid and PEG chains. Hedrick et al. [20] reported combination of ring-opening and atom transfer radical polymerization (ATRP). These authors synthesized dendrimer like multiarm poly(ε-caprolactone)-2-bromoisobutyrate which was used as macroinitiator in ATRP of 2-hydroxyethyl methacrylate and PEG-methacrylate, respectively.

So far, PEG/PEO is the choice of hydrophilic segment due to its biocompatibility and stealth properties towards protein adsorption. Nevertheless, other hydrophilic vinyl polymers are also being explored as these structural variations can induce pH and/or temperature sensitivity or create pendant sites for conjugating ligands. Also, under certain conditions, PEG can promote the aggregation of nanoparticles after freeze-drying [21]. Benahmed et al. [22] reported poly(*N*-vinyl pyrrolidone)-*block*-poly(D,L-lactide) (PVP-*b*-PDLLA) micelles. Jeong and coworkers [23,24] reported the use of poly(2-ethyl-2-oxazoline) (PEtOz) as the shell-forming polymer in PEtOz-*b*-PDLLA and PEtOz-*b*-PCL. Kim et al. [25] reported thermosensitive nanoparticles comprising poly(*N*-isopropylacrylamide)-*block*-poly(L-lactic acid).

Poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) is a hydrophilic, non-immunogenic and biocompatible polymer [26]. However, PHPMA is insoluble in toluene and tetrahydrofuran, the solvents suitable for polymer-initiated ring-opening polymerization of lactide, glycolide and ε-caprolactone. Moreover, the reactivity of secondary hydroxyl groups in the PHPMA pendant chain should not be neglected at high temperatures of melt polymerization. Breitenbach and Kissel [27] reported grafting of PDLLA and PLGA chains onto poly(vinyl alcohol) (PVA) under melt polymerization conditions. ATRP of HPMA initiated with poly(butyl methacrylate)-macroinitiator resulted in poor monomer conversion and polydisperse polymer due to the competition of PHPMA amide nitrogen atom with the added ligands, for Cu(I) catalyst, used in ATRP [28].

Due to these problems, block polymers of PHPMA with biodegradable polymers were not reported so far. We recently reported PHPMA-*b*-PCL-*b*-PHPMA by free radical polymerization of HPMA in the presence of α,ω-poly(ε-caprolactone) dithiol as the macromolecular, biodegradable chain-transfer agent [29]. In this communication, we have explored this methodology to synthesize novel amphiphilic star-PCL-*b*-PHPMA using thiol terminated star-shaped PCL as the biodegradable chain-transfer agent. Star-block copolymers were characterized for their micellar characteristics and hydrophobic drug loading capacity.

2. Experimental

2.1. Materials

3,3′-dithiobis(propionic acid) (DTPA), pentaerythritol, ε-caprolactone (CL), stannous 2-ethyl hexanoate, dicyclohexyl carbodiimide (DCC), *N*,*N*-dimethylaminopyridine (DMAP), dithiothreitol (DTT), 2,2′-azobisisobutyronitrile (AIBN), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMAC), dichloromethane (DCM) and hexane were procured from Aldrich Chemical Company Inc. (Oakville, Ont., Canada). *N*-(2-hydroxypropyl) methacrylamide (HPMA) was obtained from Polysciences Inc. (Warrington, PA). Indomethacin was from Sigma (Oakville, Ont., Canada). Spectra/Por™ dialysis membranes of 6000–8000 molecular weight cut-off were purchased from Spectrum Laboratories (Rancho Dominguez, CA). AIBN was recrystallized from ethanol. THF was freshly distilled over sodium and benzophenone before use. All other chemicals were used as received.

2.2. Instrumentation

2.2.1. ^1H NMR spectroscopy

^{1}H NMR spectra of all the compounds synthesized in this work were obtained on a Bruker (Milton, Ont., Canada) spectrometer operating at 300 MHz.

2.2.2. Molecular weight measurements

Absolute weight- (M_w) and number- (M_n) average molecular weights of polymers were determined by size-exclusion chromatography (SEC) using a Waters 1525 pump (Waters, Milford, MA) equipped with differential refractive index detector (Waters 2410) and PD 2000 light scattering detector (Precision Detectors, Franklin, MA) under the following conditions. Three columns HT2, HT3 and HT4 with a very high to low molecular weight separation range were used in series. Polymer samples were dissolved in DMF (4 mg/ml) and injected in the system. The mobile phase was DMF containing 10 mmol LiBr. Flow rate and temperature were set at 1.0 ml/min and room temperature.

2.2.3. Fluorescence spectroscopy

The apparent CAC of the polymers was estimated by a steady-state pyrene fluorescence method [23,24], which is based on the shift in the (0,0) band of pyrene in the excitation spectra from 333 to 336 nm due to its incorporation in the hydrophobic core of the micelles. A 2-ml aqueous solution of pyrene (2×10^{-7} M) was added to a 2-ml polymer solution with increasing concentrations (0.015–2500 mg/l). These solutions were kept at 4 °C in the dark and stirred gently for 16 h. Their excitation spectra (band pass 1 nm) were recorded on an AMINCO Bowman Series 2 luminescence spectrometer (Thermo Spectronic, Rochester, NY) at $\lambda_{em} = 393$ nm (bandpass 4 nm). The CAC was determined from the intersection of two straight lines (the horizontal line with an almost constant value of the ratio $I_{336\ nm}/I_{333\ nm}$ and the vertical line with a steady increase in the ratio value) on the graph of the fluorescence intensity ratio $I_{336\ nm}/I_{333\ nm}$ vs. log polymer concentration.

2.2.4. Micelle size measurements

Micelle size in aqueous solutions was measured by dynamic light scattering at 90° angle to the incident beam and at 20 °C on a Coulter N4 Plus particle size analyzer (Coulter, Miami, FL) equipped with software for differential size distribution intensity analysis. The aqueous solution of polymer (0.3 or 2 mg/ml) was passed through 0.22-μm filter before size measurement.

2.3. Synthesis of star-poly(ε-caprolactone)-tetrakis-thiol (star-PCL-(SH)$_4$)

2.3.1. Step 1: star-poly(ε-caprolactone) (star-PCL)

Two grams of ε-caprolactone (17.5 mmol), 0.03 g of pentaerythritol (0.22 mmol) and 0.025 g of stannous 2-ethyl hexanoate (0.062 mmol) were placed in a Schlenk polymerization tube equipped with a magnetic stirring bar. Vacuum was applied to the tube for 10 min. Then, the tube was filled with nitrogen. This was repeated three times. Finally, the tube was closed under vacuum and immersed in an oil bath preheated to 150 °C. Polymerization was allowed to proceed for 16 h under stirring. Polymerization tube was cooled to room temperature. The polymer was dissolved in 20 ml DCM and reprecipitated in *n*-hexane. Star-PCL was isolated by filtration and dried in vacuum for 16 h. Yield: 1.62 g (85%). ^{1}H NMR ($CDCl_3$): 4.10 δ, s, 8H (−CH_2−O−CO− of pentaerythritol (e)), 4.05 δ, t, 192H (−CH_2−O−CO− of PCL (a)), 3.64 δ, t, 8H (terminal −CH_2−OH (a′) of PCL arms), 2.30 δ, t, 192H (−CH_2−CO− of PCL carbonyl (d)); 1.64 δ, m, 424H (−CH_2− of PCL methylene groups (b)); 1.31 δ, m, 200H (−CH_2− of PCL methylene group (c)). $M_w = 13\,350$. $M_n = 9840$. $M_w/M_n = 1.35$.

2.3.2. Step 2: star-poly(ε-caprolactone)-tetrakis-(3,3′-dithiobis(propionate)) (star-PCL-(DTPA)$_4$)

One and half grams of star-PCL (ca. 0.54 mmol −OH groups) and 0.6 g of DTPA (2.7 mmol, five fold excess over −OH groups) were dissolved in 15 ml THF. To this, 0.5 g of DCC (2.18 mmol, four fold excess over −OH groups) and 100 mg of DMAP (0.82 mmol) in 10 ml THF was added in one portion. The reaction mixture was stirred at room temperature for two days. It was filtered to remove precipitated dicyclohexyl urea (DCU). THF solution was concentrated in vacuum. The viscous liquid obtained was dissolved in DCM (100 ml) and allowed to stand for 1.5 h to precipitate remaining traces of DCU. The clear solution was concentrated to isolate the product which was dried in vacuum for 16 h. Yield: 1 g (58%). ^{1}H NMR ($CDCl_3$): 4.09 δ, s, 8H (−CH_2−O−CO− of pentaerythritol (e)), 4.05 δ, t, 129H (−CH_2−O−CO− of PCL (a) + terminal −CH_2−O−CO− of PCL arms (a′)), 2.92 δ, t, 24H (−CH_2−CO− of DTPA (f)), 2.72 δ, m, 17H (−CH_2−S− of DTPA (g)), 2.29 δ, t, 135H (−CH_2−CO− of PCL carbonyl (d)); 1.64 δ, m, 316H (−CH_2− of PCL methylene groups (b)); 1.37 δ, m, 167H (−CH_2− of PCL methylene group (c)).

2.3.3. Step 3: star-poly(ε-caprolactone)-tetrakis-thiol (star-PCL-(SH)$_4$)

Half gram of star-PCL-(DTPA)$_4$ was dissolved in 5 ml DMF. 100 mg of DTT (ca. 3.6 fold molar excess over disulfide groups) was added to the solution and stirred at room temperature for 24 h. The solution was poured in 1 l cold water to precipitate star-PCL-(SH)$_4$. The product was isolated by filtration washed thoroughly with water and dried in vacuum for 2 h. The product was stored at − 20 °C until further use. Yield: 0.4 g (80%). ^{1}H NMR ($CDCl_3$): 4.11 δ, s, 8H (−CH−$_2$−O−CO− of pentaerythritol (e)), 4.06 δ, t, 150H (−CH_2−O−CO− of PCL (a) + terminal −CH_2−O−CO− of PCL arms (a′)), 2.94 δ, t, 9H (−CH_2−CO− of DTPA (f)), 2.77 δ, m, 10H (−CH_2−S− of DTPA (g)), 2.31 δ, t, 135H (−CH_2−CO− of PCL carbonyl (d)); 1.64 δ, m, 333H (−CH_2− of PCL methylene groups (b)); 1.38 δ, m, 189H (−CH_2− of PCL methylene group (c)).

2.4. Synthesis of star-poly(ε-caprolactone)-block-poly(N-(2-hydroxypropyl)-methacrylamide) (star-PCL-b-PHPMA)

In a three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser, 0.1 g of star-PCL-(SH)$_4$, 1 g of HPMA (6.9 mmol) and 0.011 g of AIBN (0.069 mmol) were dissolved in 10 ml DMF. The solution was purged with nitrogen for 30 min at room temperature and the flask was immersed in an oil bath preheated to 80 °C. Polymerization proceeded for 16 h under continuous nitrogen purging. The DMF solution was poured in diethyl ether (800 ml) to precipitate the polymer, which was dissolved in 100 ml water and dialyzed against 2 l water at 4 °C for two days. The water was replaced every 12 h. The aqueous polymer solution was centrifuged at 4000*g* for 5 min to separate any unreacted star-PCL-(SH)$_4$ (very small amount observed). Then, the solution was decanted, filtered through 0.45-μm filter and lyophilized to obtain star-PCL-*b*-PHPMA. Yield: 0.8 g (72%). Typical ^{1}H NMR (DMSO-d_6) spectral data are: 4.69 δ, s, (−CH(OH)− of PHPMA (i)); 4.03 δ, s, (−CH_2−O−CO− of pentaerythritol (e)); 3.95 δ, t, (−CH_2−O−CO− of PCL main chain (a)); 3.65 δ, s, (−OH of PHPMA (j)); 2.88 δ, s, (−(HO)CH−CH_2−NH− of PHPMA (k)); 2.24 δ, t, (−CH_2−CO− of PCL (d)); 1.45–1.70 δ, m, (−CH_2− backbone of PHPMA(l) + PCL methylene groups (b)); 1.26 δ, m, (PCL methylene group (c)); 0.79–1.00 δ, d, (−CH_3 of PHPMA backbone (m) + pendant −CH_3 of PHPMA (h)).

2.5. Ellman's assay for the detection of free −SH groups in block copolymers

Ellman's reagent was prepared by dissolving 100 mg 5,5′-dithiobis(2-nitrobenzoic acid) in 20 ml 0.1 M sodium phosphate (pH 9). 1 ml of Ellman's reagent was added to 10–50 μg polymer dissolved in 1 ml 0.1 M sodium phosphate (pH 9). The solution was allowed to stand at room temperature for 20 min, and increase in the absorbance at

412 nm was measured on a Hewlett-Packard spectrophotometer (Model 89090A, Palo Alto, CA). For comparison, Ellman's test was also performed on 0–25 μg 2-mercaptoethanol, and on 50 μg polymer added to 0–25 μg 2-mercaptoethanol.

2.6. Drug loading in star-block copolymer micelles

Twenty milligrams of indomethacin and 50 mg of star-PCL-*b*-PHPMA were dissolved in 5 ml DMAC. The clear solution of polymer and drug was allowed to stand at room temperature for 30 min. Then, 0.5 ml of water was added in portions of 0.1 ml. This solution was placed in a dialysis membrane bag of 6000–8000 molecular weight cut-off and dialyzed against 2 l water for 24 h at room temperature. The water in the outer chamber was replaced every 12 h. The solution in the dialysis bag was passed through a 0.22-μm filter and lyophilized to obtain drug-loaded micelles. Drug loading efficiencies was estimated by spectrophotometry after dissolving the micelles in DMF and measuring the absorbance at 320 nm.

3. Results and discussion

In view of the problems mentioned in Section 1, the objective of the present work was to synthesize amphiphilic star-shaped biodegradable block copolymers of PHPMA via free radical polymerization of HPMA in the presence of thiol-terminated star-shaped PCL as the chain-transferring agent. In the literature reports, Sato et al. [30] synthesized a variety of A–B and A–B–A type block copolymers by free radical polymerization of vinyl monomers, such as vinyl acetate, methyl methacrylate, *N*,*N*-dimethylacrylamide and acrylic acid, in the presence of mono or dithiol-terminated PEG, poly(propylene glycol), poly(methyl methacrylate), PVA and poly(styrene) as the chain-transfer agents. Inoue et al. [31] synthesized A–B type block copolymer micelles by radical polymerization of acrylic acid in the presence of thiol-terminated oligo(methyl methacrylate) as the chain-transfer agent. To the best of our knowledge, we were the first to report the use of biodegradable, macromolecular chain-transferring agent in the synthesis of linear triblock copolymers [29]. The work presented below extends this synthetic approach to the preparation of biodegradable star block copolymers.

3.1. Choice of thiolation chemistry for biodegradable chain-transfer agent

Star-PCL comprises primary hydroxyl groups at the polymer ends that could be utilized for thiolation. Carrot et al. [32] reported the synthesis of thiol-terminated PCL by reacting it first with 2,4-dinitrophenylthioacetic acid and subsequently deprotecting the end ester group by treatment with 2-meracaptoethanol and triethylamine. Sato et al. [30] described the synthesis of thiol-terminated poly(methyl methacrylate)s by polymerizing methyl methacrylate in the presence of thiol lactic acid followed by hydrolysis of lactate group. Same authors also described the synthesis of α,ω-poly(oxyethylene)-dithiol by first reacting PEG with tosyl chloride followed by its treatment with thiourea and NaOH [33]. However, in view of intended drug delivery applications, we preferred standard chemistry of thiol group introduction in proteins, i.e. derivatization of PCL end –OH groups with DTPA followed by the reduction of disulfide bonds by DTT at room temperature [34]. The reaction scheme for the synthesis of star-PCL-$(SH)_4$ is shown in Fig. 1. Further, it was decided to use this tetra-functional chain-transfer agent in free radical polymerization of HPMA as represented schematically in Fig. 2. In the following sections we describe the synthesis and characterization of star-PCL-$(SH)_4$ and star-PCL-*b*-PHPMA.

3.2. Star-PCL

This was synthesized following the reported procedure for star-PCL by Dong et al. [35]. However, we found that higher concentration of stannous 2-ethyl hexanoate catalyst and 150 °C, instead of the reported 110 °C, were suitable conditions for CL polymerization in our case. ^{1}H NMR of star-PCL in Fig. 3 shows presence of both pentaerythritol methylene group protons (e) and PCL terminal $-CH_2-OH$ (a′) group protons with the ratio e/a′ = 0.90 (theoretical e/a′ = 1). These spectral data are consistent with those reported by Dong et al. [35] and Veld et al. [36]. Other main chain PCL proton peaks seen in the NMR spectrum are also in accordance with the literature reports [32]. The M_n calculated by ^{1}H NMR (10 900) approaches its theoretical value (9100) based on [CL]/[pentaerythritol]. Absolute molecular weight data by SEC are $M_w = 13\,350$; $M_n = 9840$; $M_w/M_n = 1.35$. These data indicate that star-shaped PCL with hydroxyl end groups was successfully synthesized by polymerization of CL using pentaerythritol and stannous 2-ethyl hexanoate as initiator and catalyst, respectively.

3.3. Star-PCL-$(DTPA)_4$

This compound was synthesized by the DCC-mediated coupling of hydroxyl end groups of star-PCL with excess of DTPA. The possibility of crosslinking during condensation is thus minimized as reactive hydroxyl groups of PCL are rapidly substituted by DTPA. However, we did observe turbidity in the DCM solution of star-PCL-$(DTPA)_4$ during the purification step. This could be attributed to a crosslinked network of star-PCL-DTPA that precipitated. It was removed by filtration, which could be the reason for low yield (50%) of this reaction. M_n of the purified product, as estimated by ^{1}H NMR, did not exhibit significant increase as compared to starting material, i.e. star-PCL.

Fig. 4 shows the ^{1}H NMR spectrum of star-PCL-$(DTPA)_4$ with two new peaks that correspond to the

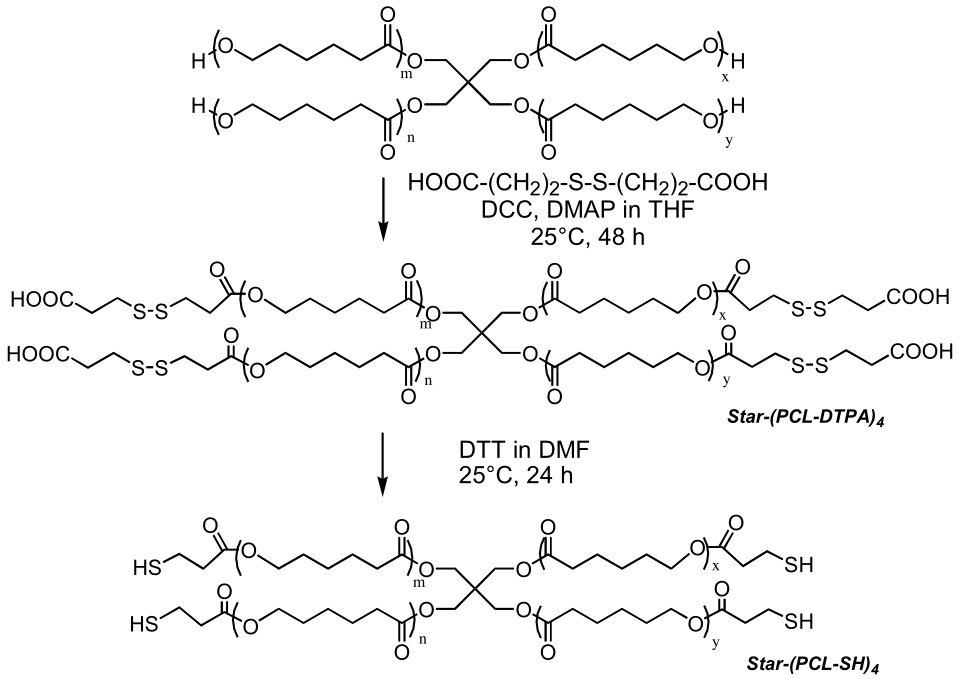


Fig. 1. Reaction scheme for the synthesis of star-PCL-$(SH)_4$.

conjugated DTPA moiety. Also, the peak for PCL terminal $-CH_2-OH$ (a′) groups have disappeared, indicating esterification of all the hydroxyl end groups. The new peaks at 2.92 δ and 2.72 δ correspond to $-CH_2-CO-$ (f) and $-CH_2-S-$ (g) moiety of DTPA conjugated at the four terminals of star-PCL. The ratios of pentaerythritol methylene protons (e) to DTPA protons (f and g) are close to their respective theoretical values, as shown in Fig. 4. Considering the polydispersity of star-PCL ($M_w/M_n = 1.35$), the data indicate 80 ± 10% esterification of star-PCL with DTPA.

3.4. Star-PCL-$(SH)_4$

The product was obtained after DTT-mediated reduction of disulfide bonds. Fig. 5 shows that the ^1H NMR spectrum of star-PCL-$(SH)_4$ is similar to that of star-PCL-$(DTPA)_4$, but with almost half the number of protons for $-CH_2-CO-$ (f) and $-CH_2-S-$ (g) groups in DTPA moiety (see details in Section 2). This clearly indicates the loss of one molecule of 3-thiopropionic acid from each arm end of star-PCL following disulfide bond reduction. Also, Fig. 5 shows that proton ratios e/f

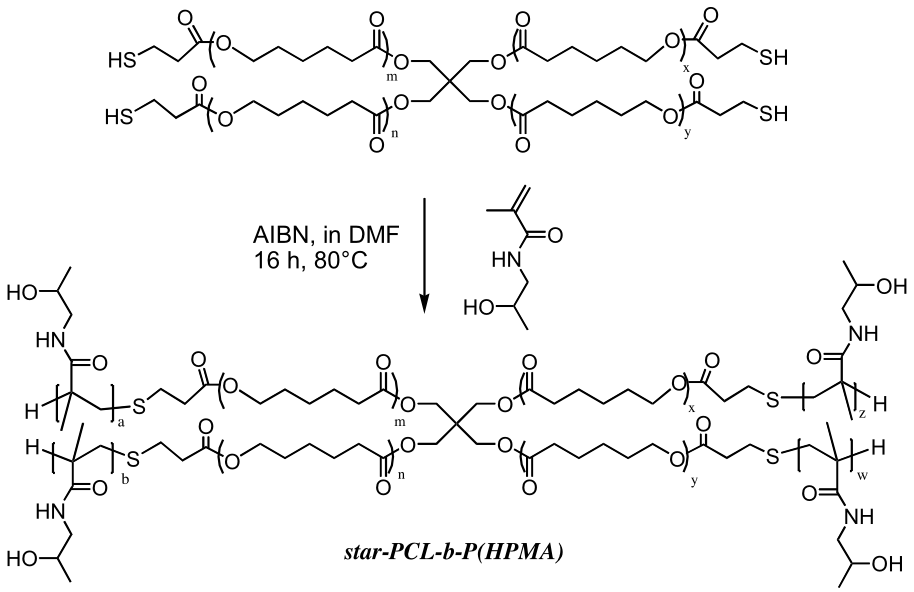
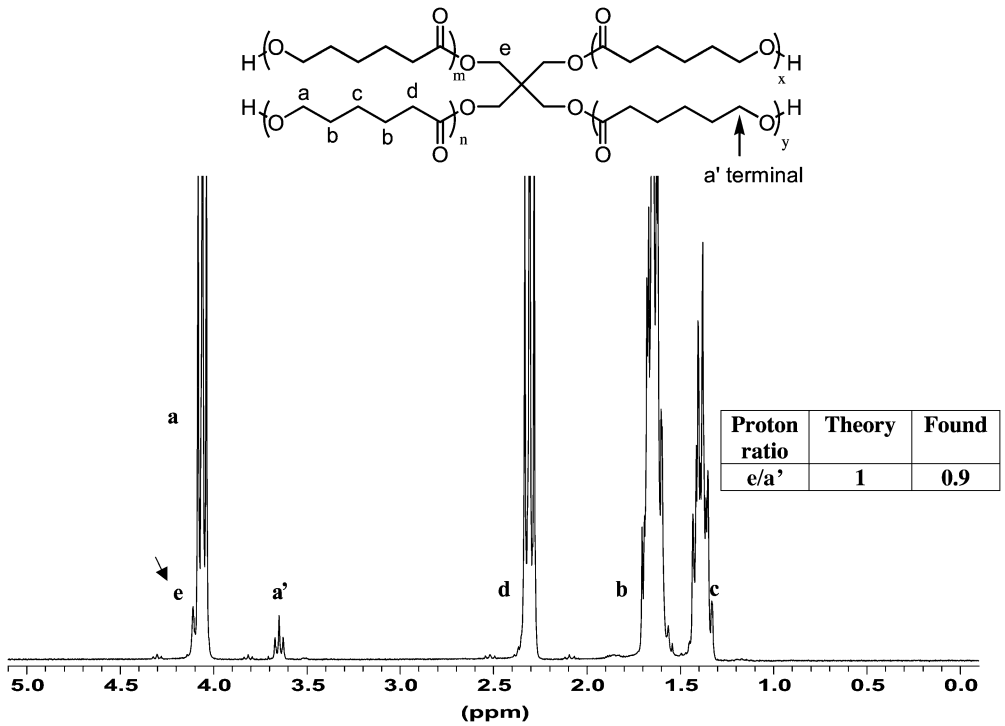


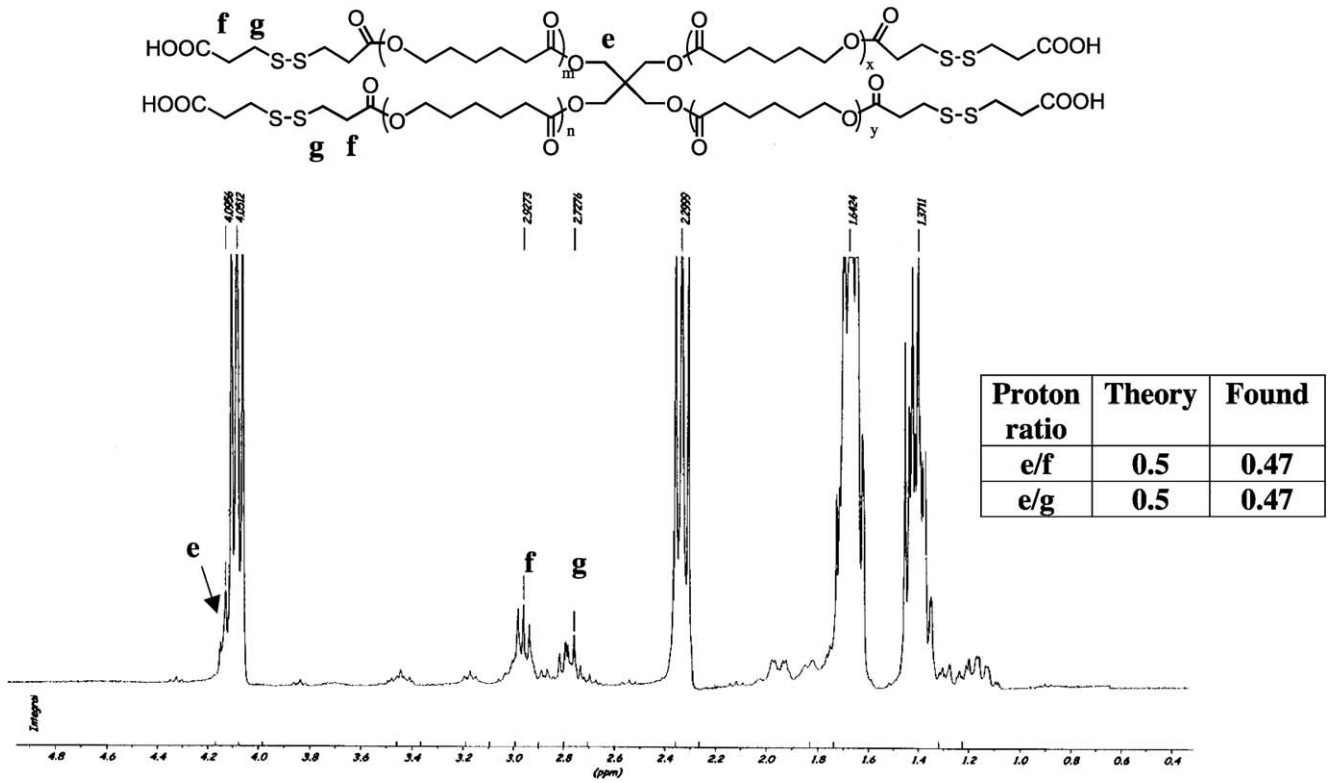
Fig. 2. Reaction scheme for the synthesis of star-PCL-*b*-PHPMA copolymers.



Proton ratio	Theory	Found
e/a'	1	0.9

Fig. 3. ^{1}H NMR spectrum of star-PCL.

and e/g are close to their respective theoretical values. These data clearly support the quantitative reduction of disulfide bonds and the formation of star-PCL-$(SH)_4$. Although PEG thiols are sensitive to air oxidation, hydrophobic macromolecular thiols, e.g. thiol-functionalized poly(methyl methacrylate) (HS-PMMA), were found to be stable [30]. In the present work also, we did not observe the formation of disulfide



Proton ratio	Theory	Found
e/f	0.5	0.47
e/g	0.5	0.47

Fig. 4. ^{1}H NMR spectrum of star-PCL-$(DTPA)_4$.

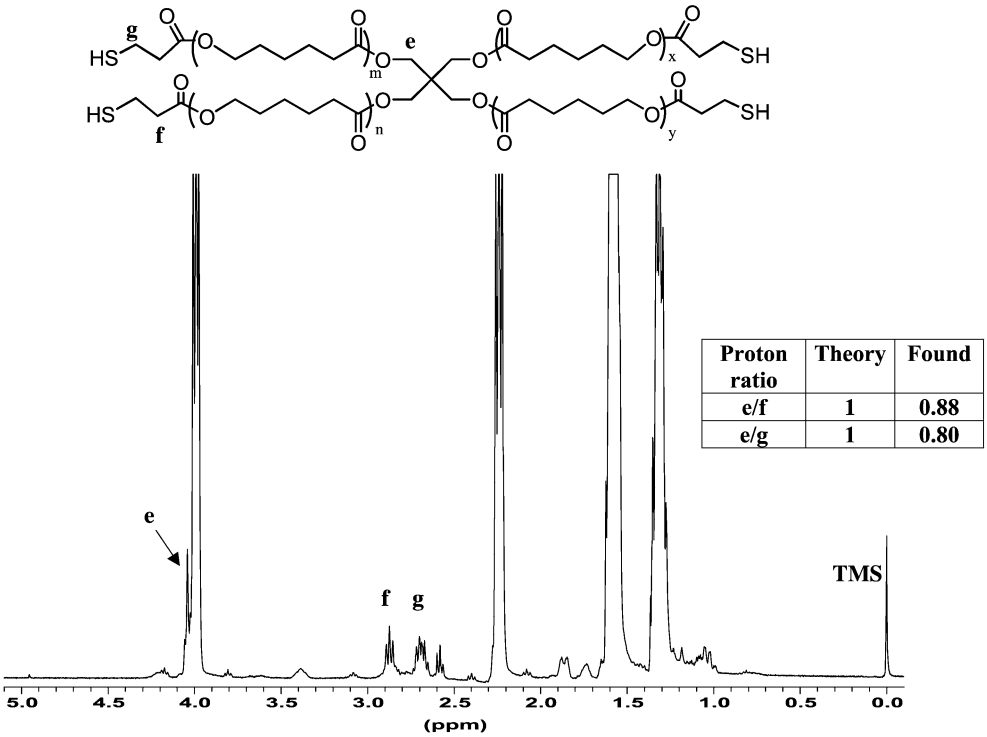


Fig. 5. ^{1}H NMR spectrum of star-PCL-(SH)$_4$.

linkages due to air oxidation during the handling of star-PCL-(SH)$_4$ and upon storage in the freezer at −20 °C.

3.5. Synthesis and characterization star-PCL-b-PHPMA

As described in Section 2, we polymerized HPMA using AIBN and star-PCL-(SH)$_4$ as the initiator and the chain transferring agent, respectively. In this procedure, AIBN initiated radical polymerization should form a small amount of PHPMA. However, after the proton abstraction by growing PHPMA chains and/or AIBN from star-PCL-(SH)$_4$, the high reactivity of thio radicals is expected to initiate new PHPMA chains and result in star-block copolymer. This is described in the following sections.

Polymers with increasing molar feed ratios of CL to HPMA (0.08–0.20) (6–14% (w/w)), were synthesized and purified as described earlier. Increasing the feed of star-PCL-(SH)$_4$ above 20% (w/w) resulted in polymers with poor water solubility. Therefore, the polymers were synthesized with a narrow difference among the feed ratios of star-PCL-(SH)$_4$/HPMA. A typical ^{1}H NMR spectrum of star-PCL-*b*-PHPMA (0.2:1), in Fig. 6, shows well-separated peaks for −O*H* (j), −C*H*$_2$− (k) and −C*H*(OH)− (i) in HPMA at 3.65 δ, 2.88 δ and 4.69 δ, respectively. Also, the peaks for pentaerythritol −C*H*$_2$−O−CO− (e), PCL −C*H*$_2$−O−CO− (a), PCL carbonyl −C*H*$_2$−CO− (d) and −CH$_2$− (c) methylene group protons are seen at 4.03 δ, 3.95 δ, 2.24 δ and 1.26 δ, respectively. The peaks for other protons in the star-block copolymer that are merged with one another, i.e. (l + b) and (h + m), are also seen in the spectrum. The proportion of CL (mol%) incorporated in the star-block copolymers was estimated from the ratio of the number of protons under the peaks characteristic of PCL and PHPMA blocks in ^{1}H NMR spectra of the polymers.

Table 1 shows that a slightly higher (0.09–0.25) molar ratio of CL/HPMA (7–17% (w/w) PCL) was incorporated in the polymers, than that used in the feed. We believe this was due to high HPMA/CL ratio which caused almost complete consumption of the chain transfer agent, but not of the excess monomer (Yields: 50–75%). Absolute molecular weights of the polymers were estimated by SEC. As mentioned earlier, since there is narrow difference in the feed ratios of star-PCL/HPMA, polymers with M_w 27 600–24 100 were obtained. Polydispersity indices of the polymers ranged between 1.65 and 1.75. All the polymers dissolved in water, indicating the absence of free/unreacted star-PCL. The polymers also dissolved easily in solvents like ethanol and methanol in which PCL is insoluble. This highlights the changed physical properties of PCL block after incorporation in star-PCL-*b*-PHPMA.

As mentioned above, chain transfer polymerization will also produce isobutyronitrile (IBN) radical-initiated PHPMA, i.e. IBN-PHPMA. Lu et al. [37] reported an elegant study on MALDI-TOF MS analysis and quantification of IBN and thiol-initiated polymer chains that form during chain-transfer polymerization of HPMA using AIBN as the initiator and alkyl thiols as chain transfer agents. We have analyzed our molar feed ratios of AIBN, chain-transfer agent and HPMA and compared these with the reported

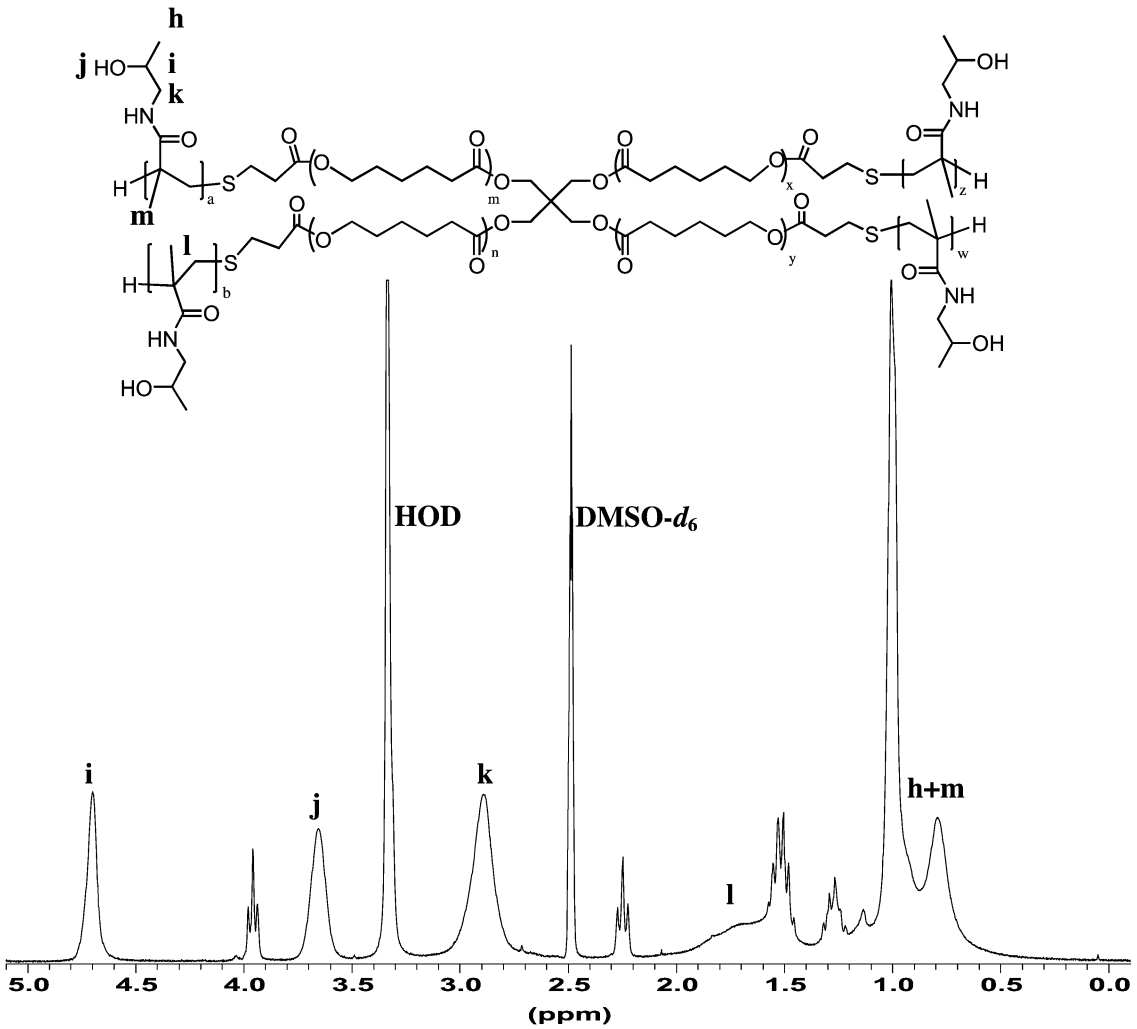


Fig. 6. ^{1}H NMR spectrum of star-PCL-*b*-PHPMA (0.2:1).

ratios. Lu et al. [37] observed that the amount of IBN-PHPMA increased predominantly with an increase in AIBN/monomer ratio as well as the reaction time. For the feed ratio of 0.05 for methyl 3-mercaptopropionate/HPMA, the amount of IBN-PHPMA increased from 5 to 16% as AIBN/HPMA ratio increased from 0.01 to 0.04 (24 h polymerization period). In our case, star-PCL-thiol groups/HPMA ratios are 0.004–0.01 and AIBN/HPMA ratio is 0.01, which is kept the same for all polymers. It will be difficult to predict the amount of IBN-PHPMA that would form in this chain transfer polymerization since our thiol groups/HPMA feed ratios are different, and also as observed by Lu et al. [37], IBN-PHPMA formation varies significantly depending on the chain-transfer agent used. MALDI-TOF MS analysis of our amphiphilic star polymers was not carried out because their molecular weights are ca. 25–30 kD, which is relatively high for MALDI characterization. DLS analysis of star-PCL-*b*-PHPMA (Table 1) 0.2% (w/w) concentration showed no population at <3 nm that would represent IBN-PHPMA and/or free block copolymer. At 0.03% (w/w) concentration, 15–25% population was seen at <3 nm which can be attributed to both IBN-PHPMA and block copolymer chains unassociated in micelles.

3.6. Ellman's assay to detect free thiol groups in star-PCL-b-PHPMA

Absence of free –SH groups in the star-block copolymers was confirmed quantitatively by reacting the polymers with 5,5′ dithiobis (2-nitrobenzoic acid) and monitoring the increase in the absorbance at 412 nm (Δ_{abs} 412 nm). Standard Ellman's assay for 5–25 μg 2-mercaptoethanol exhibited pronounced Δ_{abs} 412 nm (0.2–1.0 absorbance units) due to the reaction of –SH groups with Ellman's reagent and subsequent liberation of 5-thiol-2-nitrobenzoic acid. When the assays were performed using 10–50 μg star-PCL-*b*-PHPMA, there was practically no Δ_{abs} 412 nm. This indicates the absence of free –SH group in the polymer, i.e. –SH groups in star-PCL-$(SH)_4$ were consumed in transferring the chain to the growing PHPMA radical. These data strongly support the formation of four arm star-PCL-*b*-PHPMA copolymers. As an additional control, 50 μg

Table 1
Molecular weights, composition and micellar characterization data for star-PCL-*b*-PHPMA copolymers

Entry no.	Polymer[a]	CL/HPMA molar ratio in purified product[b]	M_w[c]	M_n[c]	M_w/M_n[c]	CAC[d] (mg/l)	$K_v \times 10^5$	Micelle size[e] (nm)	
								0.2% (w/w)	0.03% (w/w)
1	Star-PCL-*b*-PHPMA (0.08:1)	0.09	27 600	15 800	1.74	1.8 ± 0.4	8.1 ± 0.7	110 ± 10	110 ± 10 (75%), <3 (25%)
2	Star-PCL-*b*-PHPMA (0.12:1)	0.15	25 200	15 100	1.66	1.4 ± 0.2	6.5 ± 0.7	100 ± 20	125 ± 15 (85%), <3 (15%)
3	Star-PCL-*b*-PHPMA (0.15:1)	0.16	24 100	14 500	1.66	1.4 ± 0.3	7.8 ± 0.6	120 ± 10	120 ± 20 (85%), <3 (15%)
4	Star-PCL-*b*-PHPMA (0.20:1)	0.25	24 900	14 800	1.68	1.2 ± 0.1	5.1 ± 0.2	150 ± 25	125 ± 15 (85%), <3 (15%)

[a] Numbers in parentheses represent molar feed ratios of CL/HPMA.
[b] Determined by ^{1}H NMR from the ratio of the number of protons under the peaks characteristic of HPMA and PCL, respectively.
[c] Determined by SEC.
[d] The value is the average of two experiments.
[e] Measured by dynamic light scattering. The values reported are the average of three measurements. Numbers in the parentheses represent the percentage population of micelles of a particular size.

polymer was mixed with 2-mercaptoethanol standards and the assays were run. Polymer + 2-mercaptoethanol and 2-mercaptoethanol standards have negligible differences in their Δ_{abs} 412 nm value (for graphical data see Lele and Leroux [29]).

3.7. Micellar characterization of star-block copolymers

3.7.1. Determination of CAC of polymers

Excitation spectra of pyrene in aqueous solutions of increasing concentrations of star-PCL-*b*-PHPMA (0.0005–5 g/l) exhibited shift in the (0,0) band from 333 to 336 nm (data not shown). The same shift was observed earlier for PHPMA-*b*-PCL-*b*-PHPMA [29]. Jeong and co-workers [23,24] also reported shift in the (0,0) band of pyrene to 335 nm in the micelles of PEtOz-*b*-PCL. This is attributed to the partitioning of pyrene in the hydrophobic core of the micelles. Thus, the red shift from 333 to 336 nm was used for CAC determination of all the star-block copolymers synthesized in this work. As an example, Fig. 7 shows graph of pyrene fluorescence intensity ratio $I_{336\ nm}/I_{333\ nm}$ vs. star-PCL-*b*-PHPMA (0.15:1) concentration. The ratio $I_{336\ nm}/I_{333\ nm}$ is almost constant at low polymer concentrations but, starting from a certain concentration, it increases steadily, indicating the incorporation of pyrene into the hydrophobic core of the micelles. The apparent CAC was determined from this crossover point at a low concentration range. With further increase in the polymer concentration, the $I_{336\ nm}/I_{333\ nm}$ ratio increased to >1.5 and then finally reached a plateau. The CAC for star-block copolymers decreased from 2.5 to 1 mg/l as the proportion of incorporated hydrophobic PCL increased (Table 1). These CAC values are lower than those reported for star-PEO-*b*-PBLG (8–20 mg/l) [19]. This indicates much higher stability of star-PCL-*b*-PHPMA micelles.

3.7.2. Partition of pyrene probe in the hydrophobic core of micelles

The partition equilibrium constant (K_v) of pyrene in the hydrophobic core of star-PCL-*b*-PHPMA micelles was estimated according to the method reported by Wilhelm et al. [38] and Jeong and co-workers [23,24] (Eq. (1))

$$(F - F_{min})/(F_{max} - F) = K_v x_{PCL} C/1000\rho \quad (1)$$

where F_{min} is the average value of the intensity ratio $I_{336\ nm}/I_{333\ nm}$ in the low polymer concentration region; F_{max}, the average value of the ratio $I_{336\ nm}/I_{333\ nm}$ in the very high polymer concentration region; F, the intensity ratio $I_{336\ nm}/I_{333\ nm}$ in the intermediate polymer concentration region; C, the polymer concentration; x_{PCL}, the weight fraction of hydrophobic PCL in the block copolymer and ρ is the density of the PCL core of micelles, which is assumed to take the same value as bulk PCL (= 1.146) [23,24].

Thus, we can plot a straight line graph of $(F - F_{min})/(F_{max} - F)$ vs. polymer concentration, as shown in Fig. 8 for star-PCL-*b*-PHPMA (0.15:1), and obtain the value

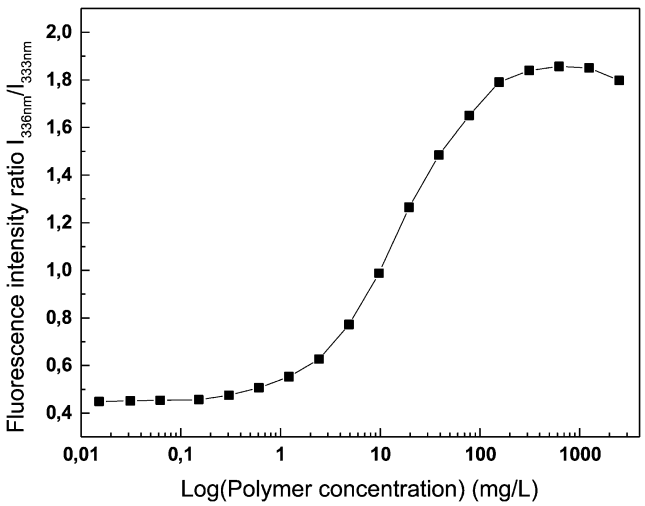


Fig. 7. Graph of pyrene fluorescence intensity ratio $I_{336\ nm}/I_{333\ nm}$ vs. polymer concentration for star-PCL-*b*-PHPMA (0.15:1).

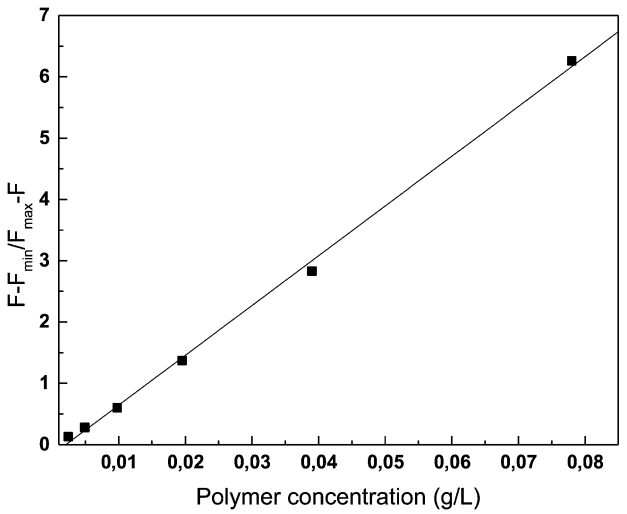


Fig. 8. Determination of the partition equilibrium constant K_v of pyrene from the graph of $(F - F_{min})/(F_{max} - F)$ vs. polymer concentration for star-PCL-*b*-PHPMA (0.15:1).

of K_v from the slope of the graph. K_v values reported in Table 1 are in the range $5–8 \times 10^5$. This is 2–4 fold enhancement over K_v values reported earlier for PHPMA-*b*-PCL-*b*-PHPMA ($K_v = 2.3–2.6 \times 10^5$) and PVP-*b*-PCL-*b*-PVP ($K_v = 3.2–4.2 \times 10^5$) [29]. Higher values of partition constants can be attributed to the higher M_n of PCL in star polymers ($M_n = 9840$) as compared to that in triblock copolymers (PCL $M_n = 2000$). This is also reflected in higher drug loading in star-PCL-*b*-PHPMA (5–12.5% (w/w), see below) as compared to that reported for PHPMA-*b*-PCL-*b*-PHPMA (2–4% (w/w)) [29]. Reported K_v values for pyrene in micelles of sodium dodecyl sulfate [39], poly(ethylene oxide)-*block*-poly(styrene) [40] and PEtOz-*b*-PCL [23,24] are 1.2×10^5, 3.0×10^5 and 5.4×10^5, respectively.

3.8. Size of the star-block copolymer micelles in aqueous solutions

Micellar size was determined by dynamic light scattering, at the polymer concentrations 0.03 and 0.2% (w/w). Table 1 shows star-PCL-*b*-PHPMA micelles exhibited highly uniform micelles of ca. 100–150 nm size. As an example, unimodal size distribution of star-PCL-*b*-PHPMA (0.12:1) is shown in Fig. 9. The observed size-range and the presence of CAC indicate these star-shape copolymers self-assemble to form micelles and thus each chain does not behave as a unimolecular micelle in solution. The micelle size remained constant even upon nine-fold dilution of each polymer solution. This indicates that the size measured here could be of individual star-block copolymer micelle and not secondary aggregates. Concentration dependant micelle size has been observed for linear block copolymers. Allen et al. [40] reported two different size populations for PCL_{20}-*b*-PEO_{44} micelles. The polymer solution of 0.02% (w/w) exhibited individual micelle with 30 nm size, whereas the population having 500 nm size was attributed to the aggregates of individual micelles. When the polymer concentration was increased to 0.1% (w/w), 30 nm size population disappeared, indicating pronounced aggregation. Large size aggregates of micelles were suggested to form due to hydrophobic association between exposed PCL cores. Similar trends were also reported by Wilhelm et al. [38] and La et al. [41]. In our earlier report of PHPMA-*b*-PCL-*b*-PHPMA, we also observed bimodal size distribution of 30 and 150 nm size micelles [29].

In the present case, the efficient steric stabilization of star-PCL-*b*-PHPMA micelles is highlighted by the fact that micelle size remained almost constant, irrespective of the polymer concentration. Moreover, molecular weight of PCL core in our case is 9840 as compared to 2300 in the case of Allen et al. [40]. Jeong et al. [9] also reported increase in the micelle size of star-PEO-*b*-PBLG from 13 to 106 nm as M_n of PBLG core increased from 4000 to 17 000.

3.9. Drug loading in star-block copolymer micelles

The ability of star-PCL-*b*-PHPMA micelles to solubilize model hydrophobic drug indomethacin was evaluated. Table 2 shows increased drug loading from 5 to 12.5% (w/w) as the proportion of star-PCL block increased in the polymers. This is comparable to indomethacin loading of 11% (w/w) reported by Liu et al. [8] in unimolecular micelles comprising dendritic phenolic core and PEG shell. Drug loading could be further improved, using a variety of other efficient loading methods reported in the literature and/or by fine tuning the polymer compositions.

4. Conclusion

Novel amphiphilic star-PCL-*b*-PHPMA was synthesized by free radical polymerization of HPMA in the presence of

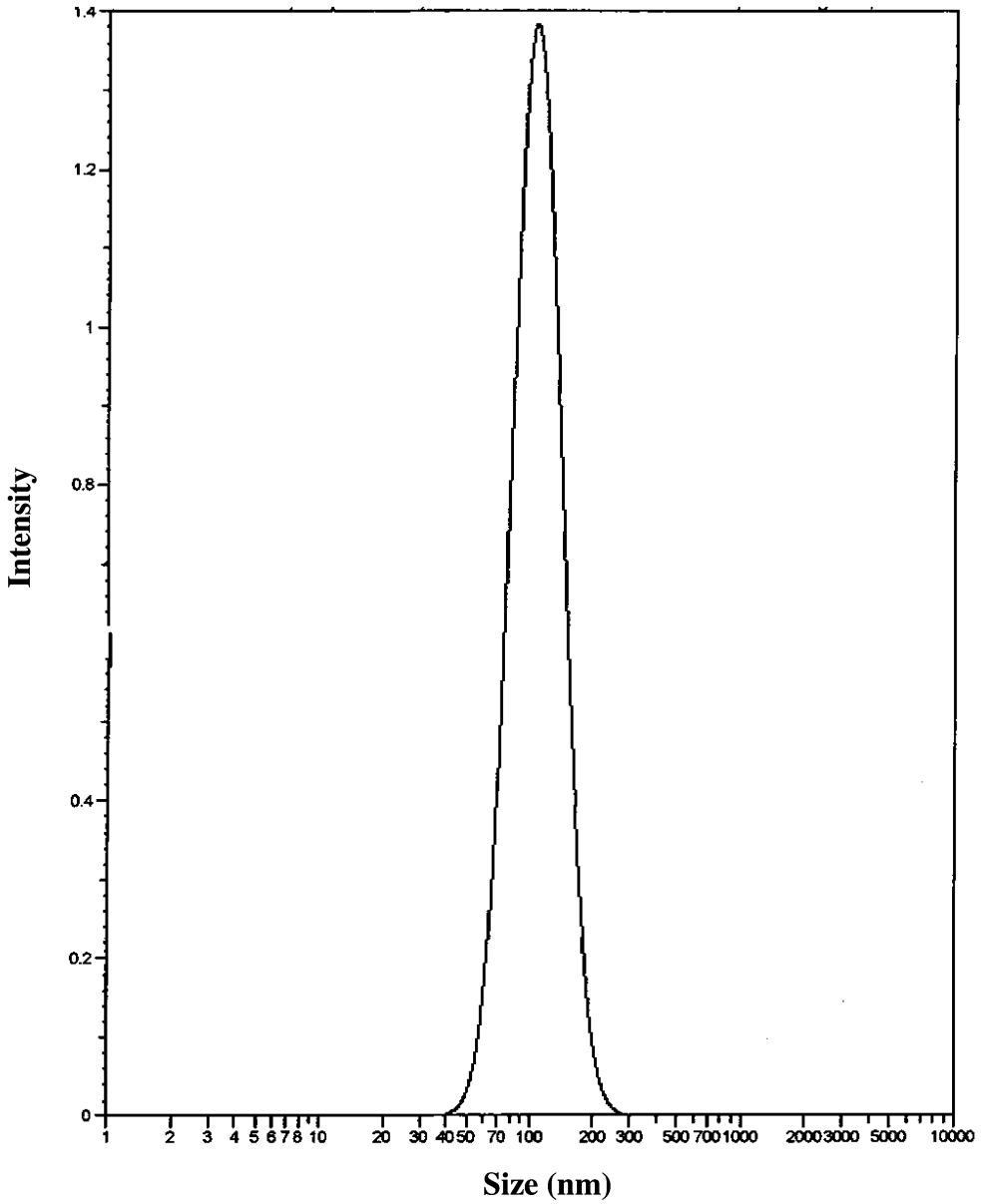


Fig. 9. Unimodal micelle size of star-PCL-*b*-PHPMA (0.12:1).

star-PCL-$(SH)_4$. This novel, biodegradable and macromolecular chain-transferring agent could find application in the synthesis of a variety of new star-block copolymers. This procedure will find advantages particularly for block copolymerization of biodegradable polymers with more than one vinyl monomers and thus creating a large number of pendant functional groups for conjugation of ligands, drug molecules and dyes. Star-block copolymers synthesized in this study self-assembled in micelles of 100–150 nm size in aqueous solutions and exhibited CACs

Table 2
Drug loading data for star-PCL-*b*-PHPMA micelles

Entry no.	Polymer	Initial indomethacin loading (w/w %)[a]	Indomethacin loading after dialysis (w/w %)[a]	Entrapment efficiency (%)
1	Star-PCL-*b*-PHPMA (0.08:1)	28.5	5.1	17.8
2	Star-PCL-*b*-PHPMA (0.12:1)	28.5	5.7	19.9
3	Star-PCL-*b*-PHPMA (0.15:1)	28.5	9.9	35.0
4	Star-PCL-*b*-PHPMA (0.20:1)	28.5	12.5	43.8

[a] Drug loading based on w/w drug/(polymer + drug).

ranging from 1 to 2.5 mg/l. Polymeric micelles also exhibited 5–12.5% (w/w) loading of hydrophobic model drug indomethacin. Such micelles could prove useful for the solubilization and targeting of various hydrophobic drugs.

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